



HEMAPROMPT FG

A Guaiac Test for Fecal and Gastric Occult Blood For use by Medical Professionals

INTENDED USE

HemaPrompt FG is a guaiac-based in-vitro slide method for the qualitative detection of occult blood in feces and gastric aspirate or vomitus by medical professionals only. It is not designed to be sent home with the patient for self-application.

For fecal testing, it is a useful aid in the diagnosis of a number of gastro-intestinal disorders, and is recommended for use in 1) routine physical examinations 2) routine hospital testing 3) screening for colorectal cancer or gastro-intestinal bleeding from any other source.

For the testing of gastric contents it is used for the early detection of occult blood in conditions such as gastric trauma, gastric or duodenal ulceration, gastric cancer, esophageal varices, situations of likely exogenous or endogenous gastritis, leukemia, and hereditary telangiectasia. These conditions may be encountered in the emergency room, recovery room or intensive care.

SUMMARY and EXPLANATION

The guaiac-peroxidase reaction, initially described by Van Deen [1], when adapted to the slide format, has been a widely used method for the detection of fecal occult blood, which is a sign of many gastro-intestinal disorders, and signals the necessity of follow-up by other diagnostic methods. Guaiac slide tests overcome the instability of guaiac solution and the hypersensitivity of benzidine and ortho-tolidine.

The same reaction has also been used for the detection of occult blood in gastric contents (when suitably buffered as with HemaPrompt FG) indicative of a number of disorders such as gastritis, ulcer, or cancer. Blood is normally absent in the stomach, and when present secondary to benign ulcer will disappear with adequate therapy, but if persistent during medical treatment, malignancy is to be suspected [19].

HemaPrompt FG provides a method of testing for the presence of occult blood in which a thin smear of feces or gastric contents to be tested is applied to the guaiac paper window. The convenience of HemaPrompt FG is that the developing solution is contained on each individual test card in a pre-measured quantity, and is applied **automatically** to the buffered guaiac paper by pulling the tab on the card and unaffected by pH in the range of 1.0 - 7.0. The application of developer solution from a separate dispenser bottle is thus avoided. In addition, a monitor printed on the guaiac paper indicates if the chemicals are functioning correctly. HemaPrompt FG results cannot be considered conclusive evidence of the presence or absence of GI bleeding or pathology. False positive/negative reactions are known to be caused by a person's particular diet or medications (see Patient Preparation). The test is intended as a preliminary indicator and not as a replacement for other diagnostic procedures such as gastroscopy, sigmoidoscopy, barium enema, and x-ray studies.

PRINCIPLE

The use of guaiac as a test for the presence of blood is based on the oxidation of phenolic compounds present in guaiac to quinones, resulting in the production of a blue color [2]. If blood is present, the heme portion of the hemoglobin (Hgb) molecule can function in a pseudoenzymatic manner, catalyzing the release of oxygen from hydrogen peroxide, which in turn causes the oxidation of guaiac.

HemaPrompt FG is a version of the laboratory guaiac slide test for fecal or gastric occult blood, and is composed of guaiac-impregnated paper buffered and mounted on a cardboard frame which permits sample applications to one side with development and interpretation from the reverse side. Feces or gastric contents containing occult blood contacts the guaiac impregnated paper and a pseudoperoxidase reaction occurs when developing solution is brought into contact with the guaiac paper, by pulling the tab.

The test paper will turn blue in 60 seconds in the presence of more than 2 mg hemoglobin/ gram of feces, and will gradually fade in 5 - 15 minutes.

It will also turn blue in the presence of more than 100 mcg Hgb/ ml of gastric fluid in 60 seconds, (see Performance Characteristics). Monitors on the guaiac slide indicate if the chemicals are functioning correctly.

REAGENTS and MATERIALS SUPPLIED

Guaiac & buffer impregnated paper and developing solution are both mounted on each slide, as is a control monitor. The developing solution of 60-70% denatured ethyl alcohol and approximately 6% hydrogen peroxide is contained within a developing pad and is exposed after pulling the tab. The positive internal control monitor contains a hematoid equivalent to a minimum of 2.0 mg Hgb per gram feces, and the area surrounding this mark is a negative control area, which should remain unchanged in color.

The fecal specimen is usually applied directly by the gloved finger, from toilet paper or by applicator (as supplied).

Gastric specimens can be applied directly from the naso-gastric tube or by obtaining a specimen from the container of gastric juice by means of the applicator supplied or by a clean cotton-tipped applicator (not supplied).

There are 50 slides with applicators in each box (Catalog Number HPFG).

Important Note Current U.S. Postal Regulations prohibit mailing completed test slides in standard envelopes. HemaPrompt FG should not be sent in the mail.

QUALITY CONTROL

Each slide is equipped with internal controls to monitor the effectiveness of the chemicals and hence the test itself. The positive internal control monitor appears as a blue checkmark and must turn blue after application of developer. The negative internal control is the background behind the positive control, which should remain unchanged in color. Failure of the controls to produce the appropriate reactions is indicative of product deterioration and the test results are invalid.

STORAGE and STABILITY

HemaPrompt FG slides should be stored at room temperature (10-22°C) and should be protected from heat, sunlight, fluorescent light, U-V radiation, humidity, volatile chemicals and gases. Do not refrigerate or freeze. They are stable until the expiration date indicated on each slide, after which time the slide should not be used.

WARNING and PRECAUTIONS

HemaPrompt FG is intended for in-vitro diagnostic use only. Skin or eye contact with the developing pad which is exposed after pulling the tab, should be avoided; flush the affected area with water should contact occur. Ingestion may be fatal or cause blindness. Keep away from heat, sparks or open flame.

PATIENT PREPARATION

i) Fecal Occult Blood

In the acute situation no particular preparation will be possible, but for screening purposes a special diet as described below is recommended to decrease the possibility of false positive results. On the other hand, dispensing with such a diet for initial screening purpose may increase patient compliance but a positive result under these circumstances would indicate the need to repeat the test in which a special diet two days prior to and during the three-day test period is followed. This diet should EXCLUDE red and rare meats, horseradish, raw fruits and vegetables like broccoli, cauliflower, red radish, cantaloupe, parsnips and turnips, or other high peroxidase containing vegetables, which can cause false positive results. An acceptable diet could include cooked fruit and vegetables such as spinach and corn as well as lettuce, prunes, grapes, and apples. Cereal, and well-cooked fish and fowl are also acceptable. If any of the recommended foods are known to cause discomfort, the patient should consult his or her physician. Because gastro-intestinal lesions may bleed intermittently and blood in feces is not distributed uniformly, all patients who test positive regardless of diet should be followed up with additional diagnostic procedures.

Certain medications such as aspirin, [3] indomethacin, phenylbutazone, reserpine, corticosteroids and nonsteroidal anti-inflammatory drugs can cause gastro-intestinal bleeding and thus give positive reactions; dosages of greater than 250mg. of Vitamin C per day have been shown to cause false negative results [4], while iron containing compounds have been mentioned as a cause of false positive reactions [5]. On the advice of the physician, these medications might be temporarily discontinued for 7 days prior to and during the test period.

ii) Gastric Occult Blood

Elective collection of gastric juice is rarely conducted. False positive and false negative reactions can be caused by medications and foods, such as mentioned above. In addition, using a guaiac based test without buffering, certain histamine (H₂) antagonists such as cimetidine (Tagamet) have been shown to cause false positive reactions [16] as with iron using other guaiac based occult blood tests intended for feces, and false negative reactions can result from low pH, but because of the buffering used in HemaPrompt FG, interfering reactions at recommended dosage of ranitidine (Zantac 300mg), ferrous sulfate (USP 300mg), cimetidine (Tagamet 300mg), or antacids (Mylanta 10-20 ml) have not been observed [21].

SPECIMEN COLLECTION

Feces can be obtained by direct rectal exam, but for screening purposes stool samples should not be collected if the patient is experiencing menstrual bleeding, constipation bleeding, bleeding hemorrhoids, when rectal suppositories or medication is being used or if there are cuts on the hand. It is recommended that smears be collected from two different areas of each stool from three consecutive bowel movements as closely spaced in time as possible, [6][7], or by the physician following a rectal exam. Using toilet paper, a specimen is taken from stool smeared on the bowl and above the toilet water level, from the toilet paper used following defecation, or from a specimen caught in a clean cup. Application to the slide may be performed from the gloved finger (as after a rectal exam), applicator, or by use of the toilet paper described above. No more than 5 days (At 10-22° C) should elapse between specimen application and testing, with patients instructed to return all slides to the physician as soon as possible. Rehydration of the specimen is neither necessary nor recommended for this time interval.

Gastric Contents obtained from the naso-gastric tube or vomitus can be applied directly from the naso-gastric tube or by means of a cotton-tipped swab. The specimen by itself may be stored up to 10 days at 5°C before testing or after application to the slide may be stored up to 24 hours at room temperatures (10-22°C).

Precautions should be followed when handling patient specimens and all materials that come in contact with these specimens. These items should be handled as potentially infectious and disposed of with proper precaution. Do not allow contact with skin or mucous membranes.

TESTING PROCEDURE

After the fecal or gastric specimen has been collected, open the test card, so that both specimen windows are visible. Apply a **very thin smear** of fecal or gastric specimen to each specimen window on the test card. The specimen windows should not be completely covered with the specimen: an outer periphery of white should be left for contrasting background color. The test card may be developed immediately (or **fecal specimens** may be developed for up to 5 days after specimen application when stored at room temperature 10-22° C, **gastric specimens** may be developed for up to 24 hours after specimen application when stored at room temperature 10-22° C. Close the card and turn the card over to the reverse side. Lift the silver tab, exposing the developer pad, and grip the card where indicated. **Slowly** pull the silver tab all the way to the right and completely remove it from the test card. Results should be interpreted after 60 seconds, but before 5 minutes (weak positive results may fade after that time).

INTERPRETATION of RESULTS

For both **Gastric and Fecal** samples, results are to be read from the card through the clear plastic window 60 seconds after developer is added. ANY shade of blue color in the patient test area is to be considered a positive occult blood result. (Neither the intensity nor the shade of blue from the positive internal control monitor should be used as a reference for the appearance of positive test results) Positive results will begin to fade after 5 minutes. An absence of blue in the patient test areas indicates no detectable occult blood, and, thus a negative result.

Occasionally gastric samples applied to the slide may appear green or blue prior to application to the test card. In such circumstances, only the additional formation of blue color can be regarded as positive; a second specimen or a second opinion may be helpful if difficulty with color differentiation is encountered. Colorblind persons should not interpret the results.

The proper functioning of the test card is indicated by the positive internal control monitor turning blue and the background color remaining unchanged (internal negative control); should the internal control monitor reactions be different, the patient result is invalid. If problem persists with internal control reactions, contact Aerscher at 410-778-2957 for assistance.

TEST LIMITATIONS

Gastro-intestinal cancers, adenomas and ulcerations do not always bleed. Results cannot be considered conclusive evidence of the presence or absence of GI bleeding or pathology, and false positive or negative reactions are known to occur under certain circumstances such as a person's diet or medication (see Patient Preparation). Hydrating stool by adding distilled water may produce false positive results and is not recommended.

In testing gastric juice in overdose situations, excessive iron compounds and certain H₂ blockers (e.g. Tagamet) could produce false positive reactions, and excess antacids or ranitidine (e.g. Zantac) could produce false negative reactions. The false positive rate may also be affected by the method of collection. Use of a large naso-gastric tube is often seen to cause bleeding initially; and in practically all clinical situations where gastric juice is obtained, some degree of bleeding might be expected. The false negative rate has not been established. The test is not intended as a replacement for other diagnostic procedures and further testing and examination by the physician such as esophago-gastroscopy, sigmoidoscopy, barium enema, and imaging studies needs to be performed to determine the exact cause and source of the occult blood.

EXPECTED RESULTS

The use of guaiac impregnated paper for the detection of **fecal occult blood** has been extensively studied and these clinical studies indicate that guaiac impregnated slide tests yield a positive result 3-5% of the time in screening programs and the percent of false positive results lies in the range of 1-2% of persons on a controlled diet i.e. a diet excluding substances listed under patient preparation above [8][9][10], with the normal daily fecal blood loss in an adult lying in the range of 1-2 mls. of blood per 100 gram of stool [11]. Sensitivity (% of subjects with the condition being sought who test positive) is difficult to estimate, but in series of patients with known colorectal cancer, 50-87% have been reported to yield positive reactions [12][13][14]. Estimates of positive reactions with adenomatous bleeding have varied widely, and appear dependant to a degree on the size of polyp, with polyps less than 2 cms. yielding less than 5% positive reactions. [15].

The significance of **gastric occult blood** has been less extensively studied than fecal occult blood. One study [17] of 153 gastric aspirates from 50 intubated healthy adults indicated all aspirates with more than 50 micrograms of hemoglobin/ml were positive. There was an apparent overall false positive rate of 25.5% in this study of normal intubated individuals, but even using less than 25 micrograms of hemoglobin/ml as the test cut-off 11.8% showed a positive reaction. The positive rate will be affected by the method of collection. A traumatic intubation can be expected to produce some degree of bleeding. When gastric juice has been obtained immediately after intubation where some degree of bleeding is to be expected, a negative result is more important than a positive. In a similar study with HemaPrompt FG (21) of 12 patient gastric samples obtained by gastroscopy, and 8 healthy volunteer gastric samples obtained by intubation, there was a pH range of 1.0-6.5, and blood was added to provide Hgb concentrations of 20 - 500 mcg/ml. above the baseline levels of blood detected (if any).

It was found of the volunteer samples 37.5% (3/8) were positive with no added blood, 60% (3/5) were positive with 20 mcg/ml Hgb added, 87.5% (7/8) were positive with 50 mcg/ml Hgb added to the specimen, and 100% (8/8) were positive at concentrations of 100 mcg/ml Hgb added. All samples with blood added in concentrations of 200 mcg Hgb/ml or greater reacted positively, and all reacted in 60 seconds or less.

SENSITIVITY

HemaPrompt FG has a sensitivity of 2 mg or greater of hemoglobin per gram of stool, and greater than 100 mcg hemoglobin per ml gastric juice.

PERFORMANCE CHARACTERISTICS

Feces: Stool from a healthy volunteer on a diet as described in Patient Preparation above was used as a baseline specimen and assumed to contain 1 ml of blood/100G of stool which is about the normal daily blood loss of an adult (20). Blood (Hb14G/dl. by Coulter) was diluted in the stool by homogenization to provide concentrations of 0.1, 0.2, 0.4 and 0.6 grams hemoglobin per 100 grams of stool. In an anemic person with a hemoglobin of 10G/dl. these levels would be achieved with approximately 1, 2, 4 and 6 mls. of blood per 100 grams of stool. These dilutions were used to test the sensitivity of HemaPrompt FG as well as to compare the reactions to those of another commercially available guaiac slide test concurrently with HemaPrompt FG on the same specimens 4 times on three occasions over a period of twelve days, with precautions taken to avoid desiccation of the specimen. HemaPrompt FG reacted positively at total hemoglobin concentrations of 2mg/G of stool (i.e. about twice the expected normal blood loss) or greater, in 60 seconds, with no reactions below this concentration. It was concluded that with stool, HemaPrompt FG reacted positively and definitely to all hemoglobin levels above 2mg/G of stool as did the other commercially available slide test which in all comparative tests with HemaPrompt FG reacted in a similar fashion.

Gastric Juice: In a comparison study (21) using samples of a synthetic gastric juice of Phosphate Buffered Saline (PBS) titrated with HCl to produce a pH range of 1.0 to 7.0, Hgb concentrations of 50 and 100 mcg/ml PBS produced 62.5% positive reactions. With 200 mcg/ml PBS and above, all samples showed a positive reaction. Similar results were seen with another commercially available test for gastric occult blood. Furthermore concentrations of ranitidine, cimetidine, ferrous sulfate, and an antacid (Mylanta) to be expected in the stomach after a maximum recommended dosing did not alter the HemaPrompt FG results. This does not necessarily apply in overdose situations when excessive iron compounds and certain H2 blockers e.g. cimetidine (Tagamet) could produce false positive reactions, and excess antacid or ranitidine (Zantac) could produce a false negative. (17)

In addition a comparison study was performed using real gastric juice samples from 20 subjects (12 patients by gastroscopy and 8 volunteers by intubation). The HemaPrompt FG results are reported under the Expected Results. Samples from each person were repeated over a two-week period. Results demonstrated excellent reproducibility at levels above 200 mcg Hgb/ml gastric juice with all samples stored up to 10 days at 5°C. There was excellent comparison to results obtained on the same samples with another commercially available buffered guaiac test. It was concluded that with gastric juice, HemaPrompt FG reacted reliably and definitely to hemoglobin levels above 200 mcg/ml. gastric juice. All monitors reacted in the expected manner. Exposing the guaiac paper to UV light for ten minutes inactivated the expected reaction.

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